

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	10624
MMRRC Strain Name	129S4/SvJae- <i>Cd86tm1Shr</i> /Mmnc
Gene Name(s)	CD86 antigen (CD86)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	1/27/14

MMRRC #10624 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

B7-2-Com-F (10624): AGATCCTAGCACCCACCTCTCCAGTACT

B7-2-WT-R (10624): CCCATGTCCTTGATCTGAACATTGTG

B7-2-Mut-R (10624): TATTGCTGAAGAGCTTGCGGC

WT reaction [B7-2-Com-F (10624) + B7-2-WT-R (10624)]

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 62°C for 30 sec

Step 4: 72°C for 30 sec

Step 5: 30x from step 2 to step 4

Step 6: 72°C for 7 min

Mut reaction [B7-2-Com-F (10624) + B7-2-Mut-R (10624)]

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 60°C for 30 sec

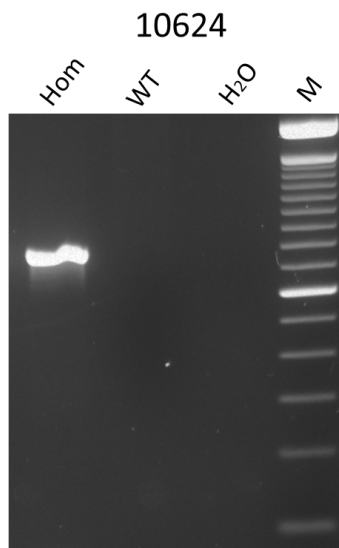
Step 4: 72°C for 30 sec

Step 5: 33x from step 2 to step 4

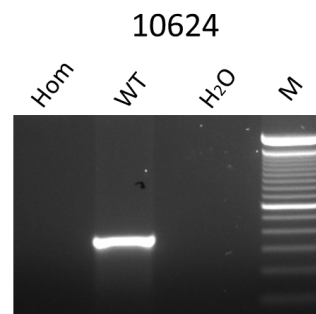
Step 6: 72°C for 7 min

Bands expected: WT: B7-2-Com-F (10624) + B7-2-WT-R (10624), 379 bp
Mut: B7-2-Com-F (10624) + B7-2-Mut-R (10624), ~750bp

Run on 2% agarose gel in TAE.



Primers: B7-2-Com-F (10624) + B7-2-Mut-R (10624)
M: 100 bp DNA ladder (Invitrogen)



Primers: B7-2-Com-F (10624) + B7-2-WT-R (10624)
M: 100 bp DNA ladder (Invitrogen)